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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Andrea	MARZIALI	Vancouver, CANADA

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Apparatus and Methods for Directing the Movement of Charged Molecules in Solution

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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OR

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 14
☑ Drawing(s) *Number of Sheets* 8
☐ Application Date Sheet. See 37 CFR 1.76
☐ CD(s), Number _____
☐ Other (specify) _____

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.
☐ A check or money order is enclosed to cover the filing fees.
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☑ No.
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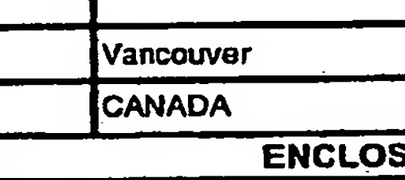
Respectfully submitted,

SIGNATURE 

TYPED or PRINTED NAME ANNARI FAURIE

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Date 30 January 2004

REGISTRATION NO. _____
(if appropriate)
Docket Number: UBC 04-023

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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Enclosures:

1. Provisional application for patent cover sheet
2. Specifications, 14 pages
3. Drawings, 8 pages
4. Credit card payment form PTO-2038 for $80.00 filing fee

APPARATUS AND METHODS FOR DIRECTING THE MOVEMENT OF CHARGED MOLECULES IN SOLUTION

INVENTOR: Andre Marziali

FIELD OF THE INVENTION

The invention relates generally to methods and apparatus for directing the movement of charged molecules in solution.

BACKGROUND TO THE INVENTION

The electric fields present during conventional direct current (DC) electrophoresis are divergence free. This limits in part the ability to concentrate and manipulate molecules by electrophoresis. Electrophoretic particle transport is typically performed in one dimension by applying a DC electric field, and is therefore limited to transport of particles toward one of the electrodes. Though this is desirable in most applications, in some it would be beneficial to have a more versatile form of electrophoretic transport. For example, it may be advantageous to transport and concentrate particles in a region that is free of electrodes to avoid electrochemical interactions between the electrodes and the particles.

Concentration and purification of nucleic acids is one such case – interaction of deoxyribonucleic acid (DNA) with the electrophoresis electrodes is undesirable and complicates the use of electrophoresis for concentration applications. Laborious and/or expensive purification methods [1 - 3] must often be employed to prepare nucleic acid samples for many biochemical assays. Though interaction of DNA with electrodes has been circumvented in some applications [4], it remains desirable to devise a completely electrophoretic method for concentration and manipulation of these charged polymers in a uniform medium, such that molecules can be transported and concentrated in regions free of electrodes.

Alternate forms of electrophoresis (reviewed in [5]) employing time varying or spatially varying fields have already been used to separate and manipulate charged polymers, including DNA. For example, it has been shown that an asymmetric alternating current (AC) waveform can cause net drift of electrophoretic particles due to non-linearity of the dependence of speed on electric field [5-7]. Dielectrophoresis [8] has also been demonstrated capable of concentrating DNA [9,10], but requires high electric field gradients to generate a significant effect.

There is a need in the art for new separation techniques that reduce electrochemistry problems that occur at electrodes, and for new methods for concentrating and transporting charged particles in one, two, or three dimensions.

SUMMARY OF THE INVENTION

We provide an apparatus and methods for directing the movement of charged molecules in solution by application of a zero-time-averaged periodic driving force, and a perturbation of the mobility or drag of the molecule that is synchronized to the periodic driving force. The drag perturbation and driving force may be applied at different frequencies, if oscillatory motion of molecules is desired. The movement of uncharged (electrically neutral) molecules may also be directed if they are carried by a charged molecule. For example, neutral proteins that interact with charged micelles may be driven electrophoretically through their interaction with the micelles. We provide examples of the invention using thermal or electrophoretic means. Optical, magnetic, hydrostatic, electroosmotic or other means of altering the mobility of the molecules may also be used. Our method offers the advantage of being able to concentrate molecules in regions defined by the perturbing mechanism. In the electrophoretic case, we have demonstrated the ability to transport DNA distributed in a gel to a region in the center of the gel, an area that is free of electrodes.

We envision the invention used to direct the movement of charged molecules in gels or liquid solutions. Example applications include, but are not limited to, DNA or ribonucleic acid (RNA) transport, purification, extraction, and concentration.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for an apparatus and methods for directing the movement of charged molecules in solution by cyclically altering the mobility or drag coefficient of the molecule synchronously with a driving force in the form of an alternating electric field , or by altering the mechanical properties of the solution synchronously with a driving force in the form of an alternating electric field.

Any effect that cyclically alters the electrophoretic mobility of a molecule can be used.

Synchronous alteration of the mechanical properties of the solution may be achieved by any means that reversibly alters the solution viscosity or density including, but not limited to, heating, cyclic dilution / concentration through evaporation, optical or magnetic control of the solution properties or orientation of its components, cyclic control of the salinity or pH of the solution and other methods??

The coefficient of drag or mobility of the charged molecule may be affected by any number of means including, but not limited to, electrophoresis, cyclic binding and unbinding to other molecules or components of the solution, optically induced conformational changes, chemically induced conformational changes, acoustically induced conformational changes , and thermally induced changes. For example, optically inducing small but reversible changes in the hydrodynamic drag of a molecule is an effective means. The electrophoretic mobility of such a molecule, or of a compound containing this molecule, could then be altered in a periodic manner by means of optical excitation. Patterning of the optical excitation over a solution using masks may be used for high resolution concentration of molecules without the need to pattern electrodes or dielectrics in the solution itself as would be required for dielectrophoretic concentration. In another example, molecules such as charged polymers in gel solutions have an electrophoretic mobility that is a function of electric field strength. The mobility of such molecules may be altered simply by changing the amplitude of the electric field they are exposed to. In a further example, molecules that bind and unbind to components of a gel solution display an electrophoretic mobility that depends on the strength of the electric

field they are exposed to. The mobility of these molecules may also be altered simply by changing the amplitude of the electric field they are exposed to.

Solutions that may be employed in this method include, but are not limited to, acrylamide, linear poly-acrylamide, POP (Perkin Elmer), or agarose gels, entangled liquid solutions of polymers, viscous or dense solutions, solutions of polymers designed to bind specifically to the molecules whose motion is being directed, simple aqueous solutions and others. For some molecules and methods for perturbing mobility or drag coefficient, the effect will be nearly independent of the solution and water may be used. Polymer gels are the preferred embodiment of the solution.

Charged molecules that may be used in this method include, but are not limited to, DNA, RNA and polypeptides.

Our method may be employed in two-dimensions sequentially on one, two or three orthogonal planes, or by using electrodes lying substantially in a common plane to create an in-plane focusing effect, and applying DC voltages on electrodes above and below the centre of the plane to direct molecules that lie outside the plane back toward it. Such a DC field would cause a de-focussing effect with respect to movement within the plane, but this could be designed to be weaker than the focusing effect of our method, such that the net result would be focusing in all three dimensions.

Our method may also be simply used in two dimensions, where molecules are confined in a third dimension by the physical extent of the solution they are contained in.

Our method may also be simply used in two dimensions, where molecules are not confined in a third dimension in order to form a concentrated line of molecules rather than a point.

Our method may also be simply used in two dimensions, where molecules are not confined in a third dimension but the rate of diffusion in this dimension, at useful concentrations of the molecule, is slower than the rate of concentration in the two dimensions.

Example 1

Electrophoretic Method

Perhaps the simplest useful implementation of our method is purely electrophoretic. By exploiting the small variation of charged polymer mobility with increasing electric field, our method allows polymers such as DNA to be concentrated in a uniform homogeneous medium. This is relatively simple, inexpensive, and of potential utility to various biochemical purification processes.

Electrophoretic transport using our method was performed by preparing an agarose gel in a custom square gel boat, preparing and inserting four separate spots of DNA into the gel, and then applying the appropriate potentials to four electrodes built into the four sides of the gel boat, to produce an approximation of uniform and quadrupole electric field patterns (see Figure 2).

The gel consisted of 8 – 11 mL of 0.25% agarose gel (Agarose 2125, OmniPur, EMD Chemicals, Gibstown, NJ) in 0.1X TAE buffer. DNA was visualized by adding Ethidium Bromide (12 μL at 500μg/mL) to the gel and imaging it under UV light.

The gel boat formed a 3.8 cm square over a UV transparent acrylic base. Four gold electrodes extended across one third of each side of the gel boat, approximately 2.5mm from the bottom of the gel boat. The electrodes were submerged in the gel. DNA was prepared by mixing 8μL of 500μg/mL λ phage DNA (48,502bp, part no. N3011L, New England Biolabs, Beverly, MA) with 12μL of 0.1 X TAE. The DNA was pipetted directly into the gel once the gel was set, and arrayed as a diamond of four spots each approximately the same distance (0.5 to 1.5 cm) from the estimated center of the electrode pattern. Each of the four spots consisted of 5μL of the above mixture. A thin covering of 0.1 X TAE was placed on the gel after the DNA was inserted and the covering was maintained over the run.

The electrodes were connected to Kepco (Flushing, NY) BOP 200-1M amplifiers that were in turn controlled by LabVIEW (National Instruments, Austin, TX) software and a data acquisition card (NI PCI-6711, National Instruments).

To simulate the electric fields without requiring analog control of the applied potentials, sinusoidal voltages were approximated by discrete forms, as shown in Figure 3. Each period of the sinusoid was represented by 12 discrete voltages, leading to 12 distinct sets of potentials applied to the electrodes during our electrophoretic method.

The quadrupole component of the field was formed by applying equal potentials to opposing electrodes, and opposite potentials to adjacent electrodes. Table 1 lists the relative potentials at each of the four electrodes of Figure 2, given discrete approximations of the uniform field and the quadrupole field. Note that the oscillation frequency of the quadrupole field is twice that of the uniform field as required. As in Figure 2, electrodes B-D lie in the x direction, and A-C lie in the y direction. Uniform field components in Table 1 are denoted by the suffix "u", while the quadrupole field components are denoted by "q".

To simplify the electronics, one of the electrodes, arbitrarily picked to be electrode C, is set to zero potential for all time intervals, and the other electrode potentials are shifted accordingly. Performing this transformation and scaling the potentials by the actual applied voltage leads to the final form of the applied potentials listed in Table 2. Each pattern of potentials was applied to the electrodes for one second, thus cycling through all patterns every 12 seconds.

Numerical Simulation of Particle Motion

A numerical simulation of particle motion under potential pattern using our method of Table 2 was performed using LabVIEW. A 2-D solution for the potentials in the plane of the gel was found for each electrode pattern by using a finite difference method in Matlab 6.5.1 (The Mathworks, Natick, MA). Potentials were found on a 60 x 60 matrix representing the gel, with appropriate boundary conditions for the edges of the gel exposed to the electrodes. The LabVIEW code performs a simulation of particle motion based on an empirical function for the particle mobility, and the dependence of this mobility on electric field. The electric field at the position of each particle in the

simulation is arrived at by finding the gradient of a fourth order polynomial fit to the potential in the neighbourhood of the particle.

Parameters for the simulation were set to the measured values $\mu_0 = 1.4 \times 10^{-9}$ m^2/Vs, $k = 2.5 \times 10^{-12}$ m^3/V^2s, with $\Delta t = 100$ ms, and the potentials as described in Table 2.

Figure 1 shows a numerical simulation of the particle path for a system in which the magnitude of the rotating fields and quadrupole fields are comparable; similar paths are taken by the particle regardless of its azimuthal starting angle with respect to the centre of the electrode system.

Mobility Measurements

To make appropriate λ phage DNA mobility measurements, the same gel concentration and electrode separation were used as in the regular experiments using our method, except a larger gel boat (C.B.S. Scientific, CBMGU-202T, Del Mar, CA) was used for ease of measurement. The same computer/amplifier connection was also used.

Two gold electrodes were glued to the bottom of the gel boat across its width at a separation of 3.8cm. A gel of the same thickness and concentration was poured and two 5μL samples of λ phage DNA were inserted ~ 2 mm from the ground electrode. The other electrode was connected to a Kepco BOP 200-1M power supply.

A custom LabVIEW program was written that raised the voltage of the connected electrode by 25V in 2-minute intervals (from 0-200V). The timer on the camera was synchronized to take a picture after every voltage increase.

The DNA velocity was obtained from successive images, and plotted against the electric field to obtain mobility.

Results

An electrophoretic demonstration of transport and focusing using our method was performed using the equipment and methods described above. DNA was chosen for this example partly because of the well-known non-linear dependence of its drift velocity on electric field. Figure 4 illustrates transport of four DNA spots toward the center of the electrode pattern, resulting in transport of all four spots into a single concentrated central spot.

The motion of a particle in the experimental test of our method can be estimated by analyzing the motion of the intensity centroids of the four DNA spots in Figure 4 and other similar experiments. This estimate relies on the approximation that our effect is approximately constant over the spot area – though this is not strictly correct, we found that, after the initial motion, spots change very little in morphology and size prior to interaction with other spots, and therefore it appears that the entire spot can be reasonably treated as a single particle.

Figure 5 is a plot of measured DNA velocity as a function of electric field strength for the DNA and conditions used in our demonstrations.

Figure 6 shows a comparison of measured DNA spot distance from the origin as a function of time, compared to numerical and analytic predictions. The spot position is averaged over all spots visible in a given time interval. Spot trajectories for spots starting at different radial distances from the origin are shifted in time such that the start time for trajectories starting closer to the origin is replaced by the time at which the other trajectories reach the starting distance.

Agreement between the model and experiment is good in the regime shown in Figure 6. The analytic prediction, on the other hand, relies on a small-signal analysis of the quadrupole field and is therefore not expected to exactly predict the experimental trajectories in this case where the quadrupole field is of comparable amplitude to the uniform field. Nevertheless, it is encouraging that the form of the behavior is substantially the same in this simple analytic model.

Figure 7 is a plot of average spot velocity (time averaged over 15 minutes) as a function of radial distance form the origin, showing explicitly the linear dependence of

velocity on distance expected from the analytic model. Two curves are shown for the numerical model – one for a particle trajectory started in the x-axis, and one for a particle started at x=y=1.5 cm form the origin. The difference in the two curves as the distance from the origin increases reflects the azimuthal asymmetry in the time-averaged electric field pattern that results from the finite size of the electrodes. The numerical model and experiment agree well only for short distances to the origin, beyond which the experimentally measured velocity becomes nearly independent of distance. It is expected that at larger distance from the origin, particles experience fields greater than those explored in our mobility measurements. Since the numerical model is based on the mobility measurements, it is only valid for the range of electric field amplitudes explored in the measurements of Figure 5. Effects resulting from larger field amplitudes are not accounted for and may be responsible for the observed deviation from the model.

Although we attempted to place the DNA spots in the experiment along the x and y axes defined by the electrodes, placement of the spots may not have been exactly on axis and this may be reflected in part in the spot trajectories. Occasionally (as seen in Figure 4) spot velocities did not follow the expected symmetry – this is suspected to result from non-homogeneity in the agarose gel, as it did not occur in every experiment nor did this always affect the same spots.

Though this example is intended primarily to demonstrate the transport of DNA using our method, it is clear that DNA, and other charged molecules, can be spatially concentrated using our method.

Figure 8 shows focusing of DNA from an initially dilute solution of 0.25% agarose and lambda phage DNA. The DNA concentration in the final central spot is over 3,000 times more concentrated than the initial concentration.

Observations of spot radius (FWHM/2) from the experiments yields limiting radii on the order of ~ 500 μm. Continued focusing leads to a decrease in fluorescent intensity, likely through diffusion of DNA in the z direction into the buffer above the gel. It is conceivable that in conditions where such diffusion was inhibited, smaller spot sizes and higher concentrations could be achieved.

We have recently shown that focusing occurs in agarose gel that is sandwiched between two plastic sheets, such that molecules cannot escape the gel. Other means of confining the DNA to the gel include sandwiching the gel between layers of denser gel, or submersing the gel in oil. In some cases, buffer or oil must be cooled and re-circulated to dissipate heat from the gel. In the case where the gel is encased in plastic, the plastic chip is immersed in ice water to dissipate heat.

Example 2

Thermal Method

Another simple demonstration of our method was conducted by thermally altering the drag coefficient of current-carrying solute ions in an electrolyte. A microscope slide, cover slip and epoxy were used to construct a chamber capable of holding 300μL of 2.0M NaCl solution. Two gold wire electrodes were glued to the microscope slide 1cm apart such that they were immersed in the NaCl solution. One of the electrodes was grounded and the other connected through a 1kΩ resistor to an AC amplifier. Nickel-Chromium Alloy wire (NIC60-015-125-25, Omega, Stamford, CT) was glued to one side of the microscope cover slip to allow heating of the solution.

During operation, 1.32A of current was pulsed to the heater in the form of a 50% duty cycle, square wave at 10Hz. A small fan running continuously was used to cool the microscope slide during off cycles of the heater. A 12Hz, 3.0V_{rms} sine wave was applied across the resistor and electrodes. These two signals were mixed and the output difference frequency (of 2Hz) was fed into the reference input of a lock-in amplifier (SR830 DSP, Stanford Research Systems, Sunnyvale, CA). To measure the periodic current resulting from our method, the voltage across the 1kΩ resistor was measured with the lock-in amplifier and the 2Hz component was singled out for analysis.

The driven temperature oscillation of the sample solution was measured directly by a thermocouple (0.005-36, Omega) glued to the microscope slide between the electrodes. The 2 Hz component of the thermocouple output was also analyzed using the lock-in amplifier.

Results

When applying an AC potential across an electrolyte solution, and synchronously raising and lowering the temperature of the solution, we found a net transport of ions occurred. When the oscillation frequency of the AC potential differed from the frequency of the thermal oscillations, a detectable component of the ionic current was present at the difference of the two frequencies, indicating alternating (AC) transport due to our method.

Using the equipment and procedure described here, an ionic current oscillating at 2Hz was detected. We assume the temperature dependent change of the electrolyte's resistance is small compared to both the 1kΩ current-monitoring resistor and the DC resistance of the solution.

CHARTS, DRAWINGS & FIGURES

	A electrode			B electrode			C electrode			D electrode			Totals		
Time interval	au	aq	at	bu	bq	bt	cu	cq	ct	du	dq	dt	ux	uy	uq
1	0	1	1	1	-1	0	0	1	1	-1	-1	-2	2	0	4
2	1	0	1	1	0	1	-1	0	-1	-1	0	-1	2	2	0
3	1	-1	0	0	1	1	-1	-1	-2	0	1	1	0	2	-4
4	1	-1	0	0	1	1	-1	-1	-2	0	1	1	0	2	-4
5	1	0	1	-1	0	-1	-1	0	-1	1	0	1	-2	2	0
6	0	1	1	-1	-1	-2	0	1	1	1	-1	0	-2	0	4
7	0	1	1	-1	-1	-2	0	1	1	1	-1	0	-2	0	4
8	-1	0	-1	-1	0	-1	1	0	1	1	0	1	-2	-2	0
9	-1	-1	-2	0	1	1	1	-1	0	0	1	1	0	-2	-4
10	-1	-1	-2	0	1	1	1	-1	0	0	1	1	0	-2	-4
11	-1	0	-1	1	0	1	1	0	1	-1	0	-1	2	-2	0
12	0	1	1	1	-1	0	0	1	1	-1	-1	-2	2	0	4

Table 1. Normalized discrete potentials applied to each electrode to simulate continuous potentials using our method.

Pattern	A Electrode	B Electrode	C Electrode	D Electrode
1	0V	-66V	0V	-198V
2	132V	132V	0V	0V
3,4	132V	198V	0V	198V
5	132V	0V	0V	132V
6,7	0V	-198V	0V	-66V
8	-132V	-132V	0V	0V
9,10	-132V	66V	0V	66V
11	-132V	0V	0V	-132V
12	0V	-66V	0V	-198V

Table 2. Potential patterns applied to the electrodes of Figure 2 to generate electrophoretic transport using our method. Electrode C was arbitrarily chosen as the ground electrode.

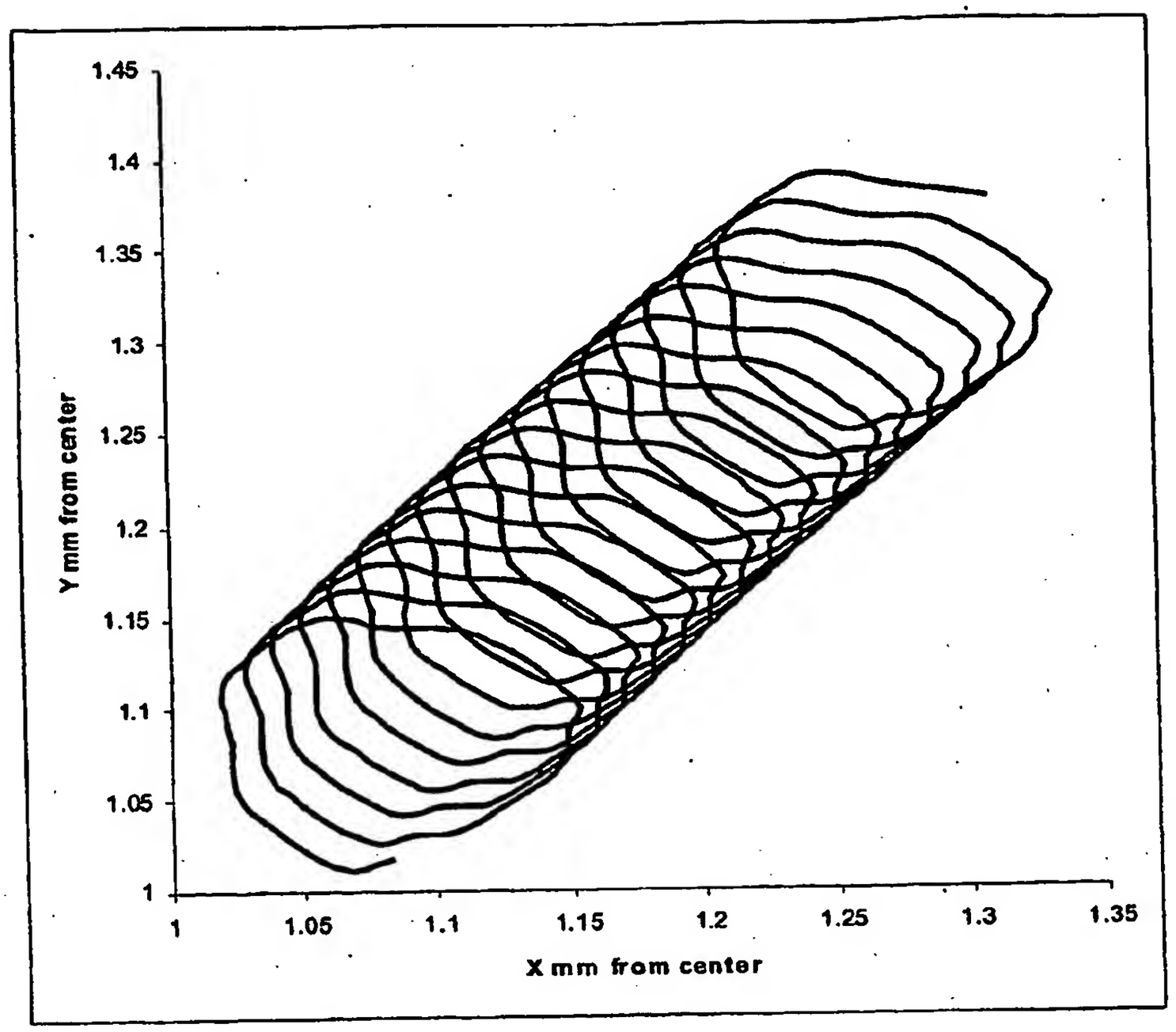

Figure 1. Numerical simulation of particle motion with uniform field rotating at angular frequency ω and quadrupole field oscillating at 2 ω. Motion begins at the top right of the path and ends to the bottom left, over 200 seconds in real time. The rotating fields have been approximated by twelve sequential configurations of potentials as described in Methods. Each configuration is applied for 1 second. One loop in the particle spiral path corresponds to a full cycle through all 12 potential configurations. The uniform field amplitude is $E = 3845$ V/m at the centre of the electrode pattern, the quadrupole field amplitude in the same location is $E_q = 4.2 \times 10^5$ V/m^2, or approximately 4200 V/m at 1mm from the center of the pattern.

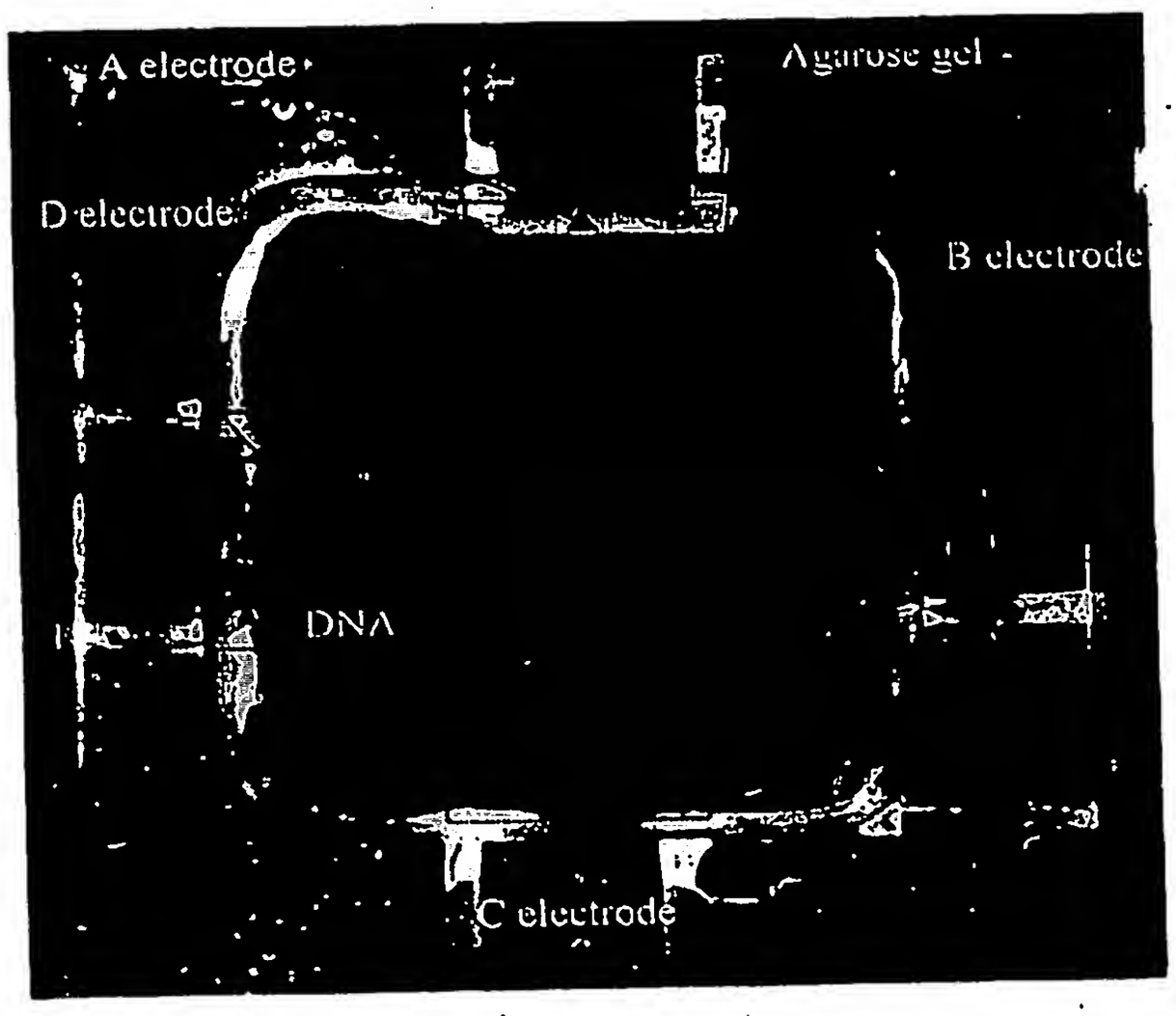

Figure 2. Photograph (under UV illumination) of the gel apparatus used for the electrophoretic method, including four DNA spots prior to transport.

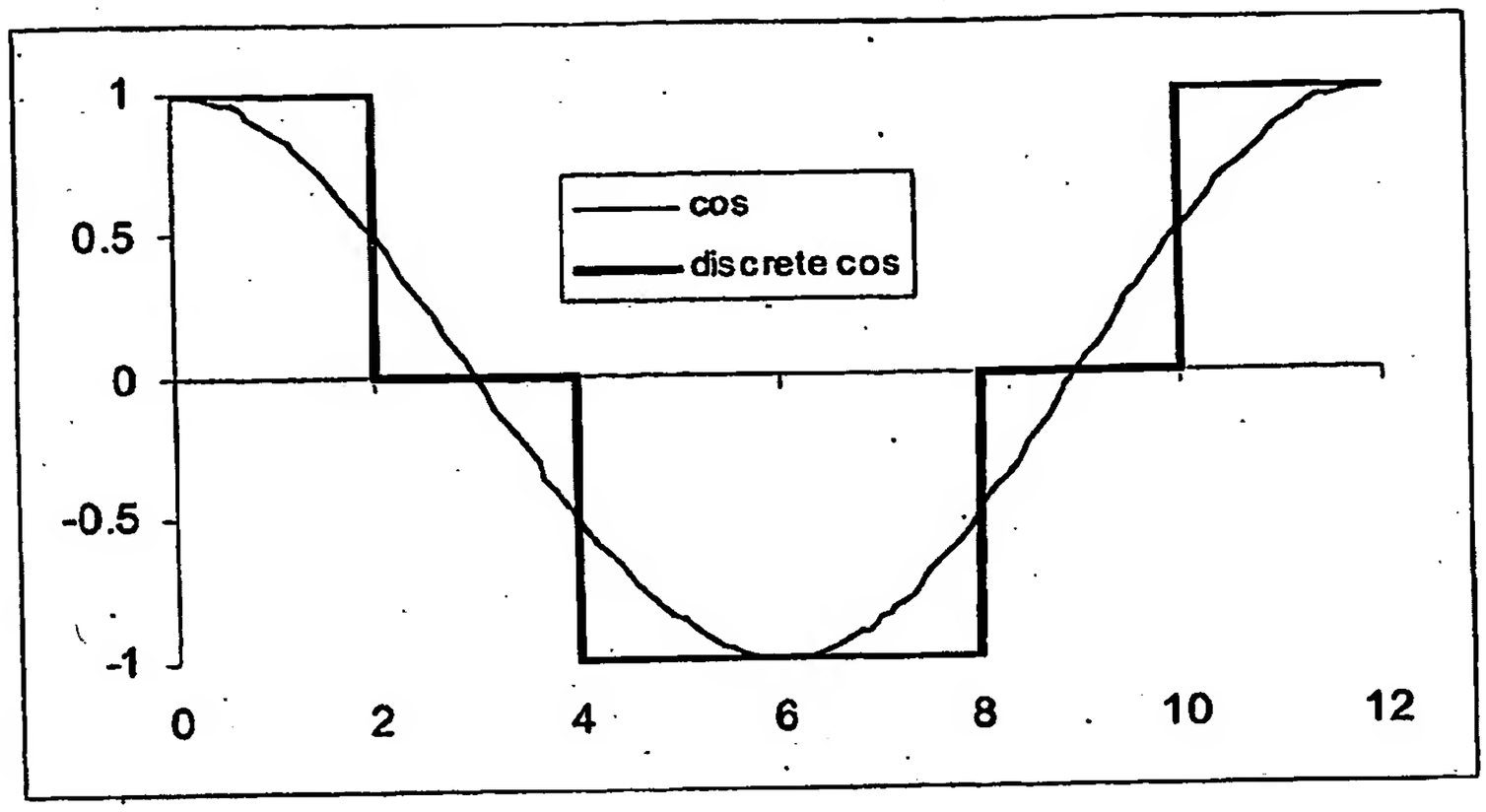

Figure 3. Discrete approximation of sinusoidal applied potentials.

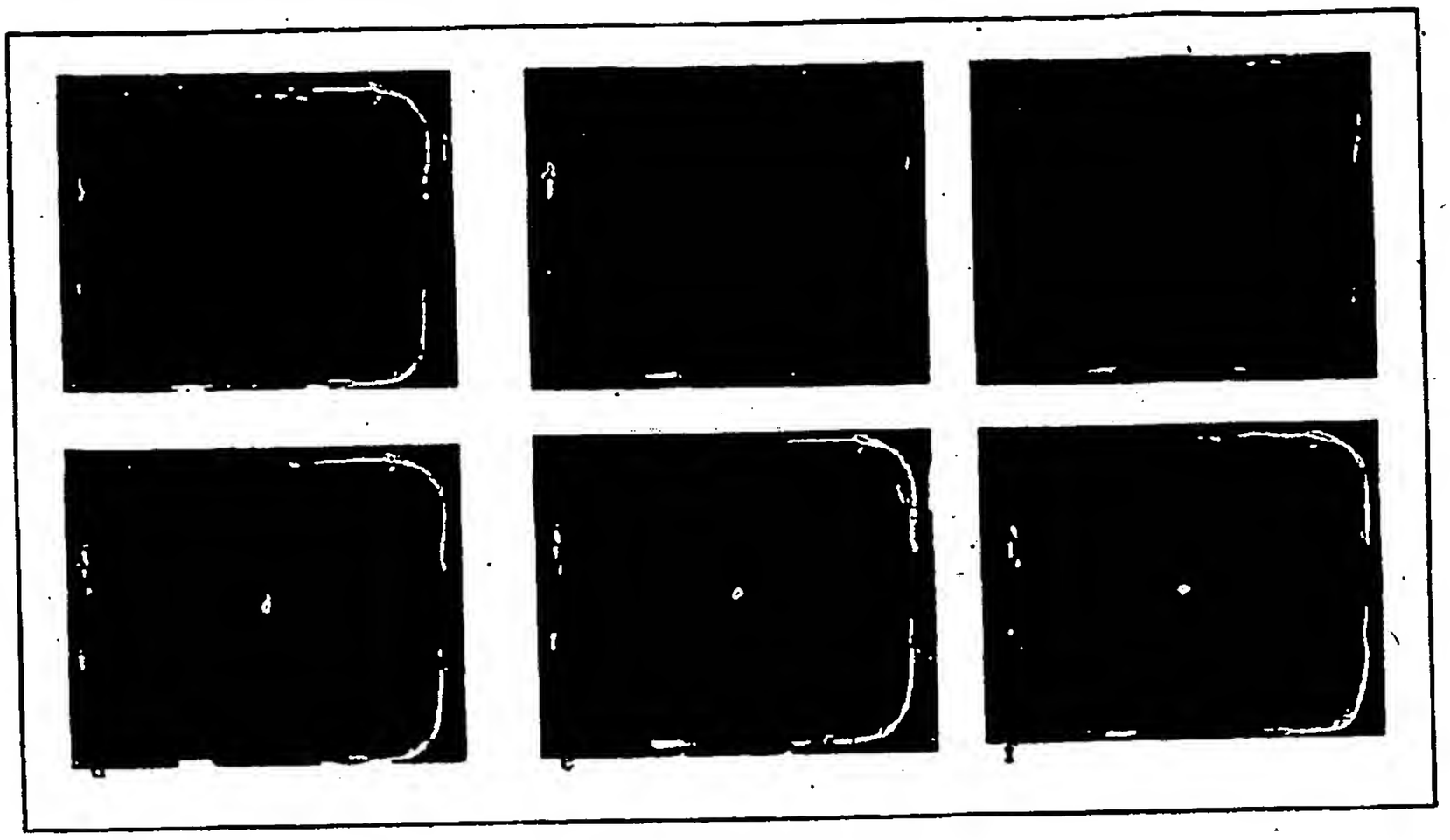

Figure 4. Electrophoretic transport of four DNA spots in an agarose gel using our method. All spots move toward the center of the electrode pattern, though the top and bottom spots initially move more slowly, possibly due to inhomogeneity in the gel. Snapshots were taken with a digital camera under UV illumination. The elapsed time between successive images is 20 minutes, and the initial spot separation is approximately 1 cm.

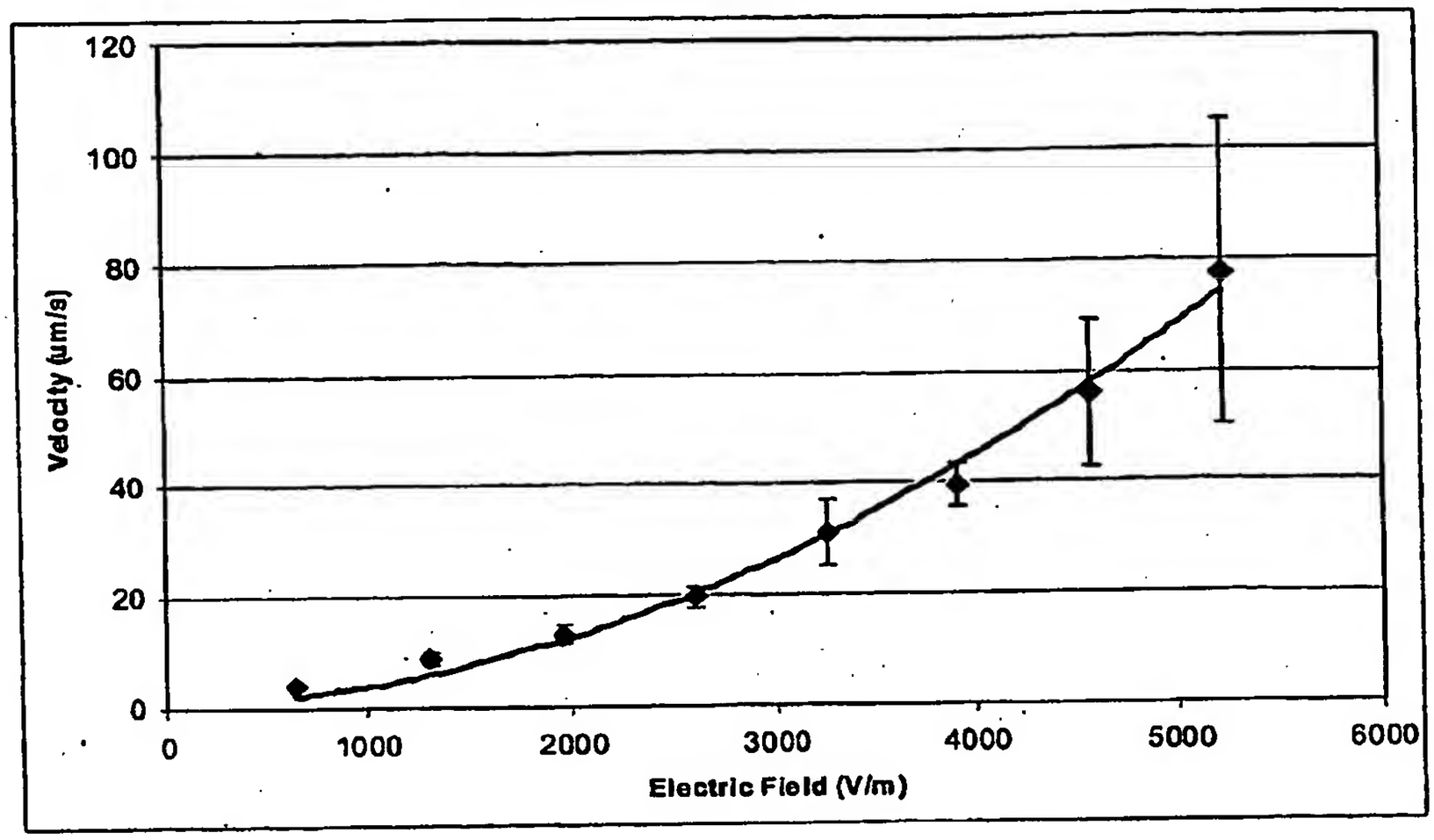

Figure 5. Measured velocity of λ phage DNA (48.5 kbp) as a function of electric field. See text for conditions. The solid line is a quadratic polynomial fit to the data.

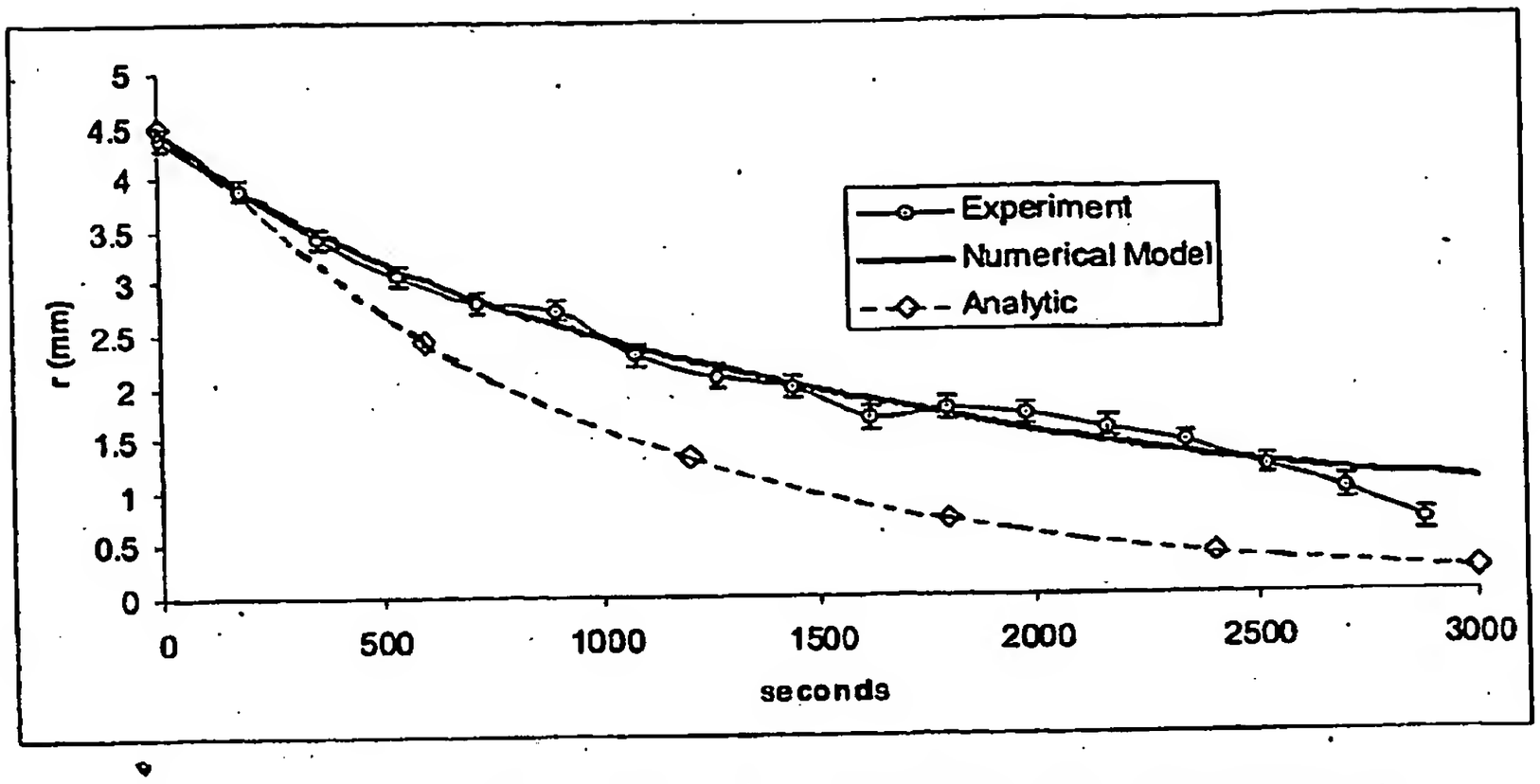

Figure 6. Averaged radial distance of DNA spots from the origin plotted from a) experimental results, b) numerical predictions, and c) analytic model. Error bars denote uncertainty in the determination of the spot centroids.

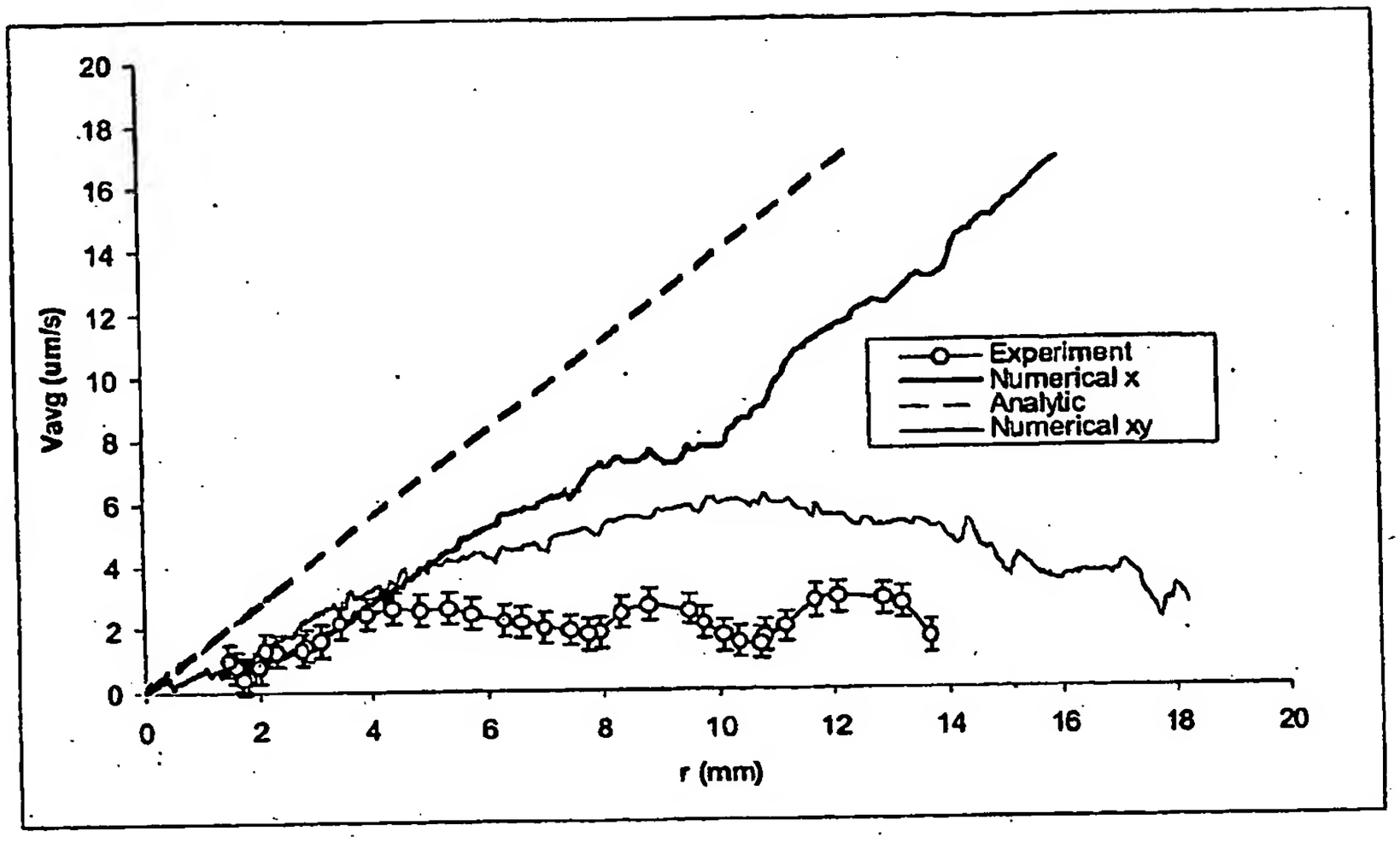

Figure 7. Average DNA velocity as a function of radial distance of DNA spots from the origin plotted from a) experimental results, b) numerical simulation (started at x = 1,5 cm, y =0).c) numerical simulation (started at x =1.5 cm, y = 1.5 cm), and d) analytic model. Experimental data below r = 1 mm is not available due to difficulty in extracting individual spot centroids once the spots begin overlapping at the center of the image. The error bars are based on uncertainty in the determination of the spot centroids.

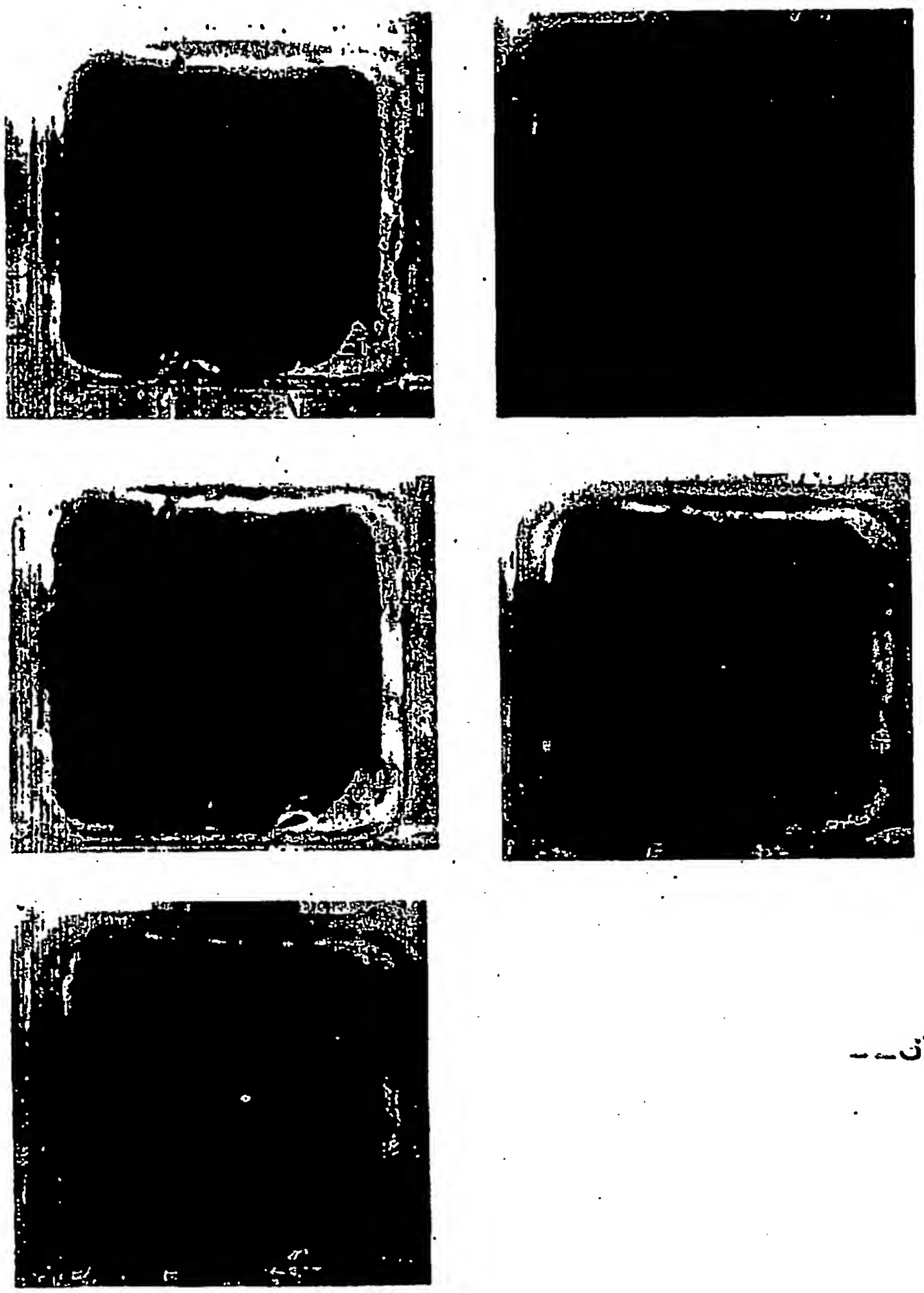

Figure 8 Concentration of lambda phage DNA from a dilute solution using electrophoretic perturbation of DNA mobility in synchrony with an alternating electrophoretic field.

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